Influence of Drugs and Nutrients on Transporter Gene Expression Levels in Caco-2 and LS180 Intestinal Epithelial Cell Lines

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Purpose. The aim of this study was to investigate the influences of various drugs and nutrients on the expression levels of intestinal drug transporters PEPT1, MDR1, MRP2 and MRP3, and drug-metabolizing enzyme CYP3A4.

Methods. Quantitative reverse transcriptase polymerase chain reaction was used to quantitate transporter and CYP3A4 mRNAs. Western blotting was used to determine protein levels of P-gp. Transport studies of P-gp were performed using cultured Caco-2 cell monolayers.

Results. The expression of MDR1 mRNA was increased by all-*trans* retinoic acid and in glucose-depleted medium, whereas little change of MRP2 and MRP3 mRNA was observed in Caco-2 cells. Substrates and inducers of P-gp or CYP3A4 tended to produce parallel changes in the expression of MDR1 and CYP3A4 mRNA in LS180 cells, whereas in Caco-2 cells no such coordinate response was observed, possibly due to the absence of the expression of steroid xenobiotic receptor (SXR) in this cell line.

Conclusion. Several drugs and nutrients were found to affect transporter gene expression level in two human intestinal epithelial cell lines. Since SXR is involved in some expression-regulatory processes, and Caco-2 cells lack SXR, LS180 cells as well as Caco-2 cells should be used for the study of the regulation of intestinal transporters.

KEY WORDS: intestinal transporter; expression level; Caco-2 cells.

INTRODUCTION

The small intestine is the major absorption site for nutrients, and various intestinal transporters have been found to transport not only nutrients but also many drugs. For example, PEPT1, an oligopeptide transporter, efficiently takes up di- and tripeptides, as well as peptide-mimetic drugs, such as ß-lactam antibiotics and angiotensin-converting enzyme inhibitors. P-Glycoprotein (P-gp) acts as an efflux pump to limit the bioavailability of drugs after oral administration. In addition to P-gp, multidrug resistance-associated proteins (MRPs) also function as efflux pumps in the intestine, and may limit drug absorption (1).

Administration of certain drugs or availability of certain nutrients has been reported to influence the bioavailability of drugs that are substrates of the intestinal transporters. After oral administration of rifampin, the plasma concentration of orally administered digoxin was decreased in healthy volunteers as a result of induction of the expression of multidrug resistance gene 1 (MDR1, coding for P-gp) in the intestine (2). Transcriptional activation of the rat PEPT1 gene by amino acids and dipeptides in the diet was reported to increase the transport activity (3), which should enhance uptake of peptide-mimetic drugs. However, in general, it remains unclear how transporter gene expression is influenced, either quantitatively or qualitatively. Therefore, it is important to investigate in detail the changes in expression levels of transporters, which may alter the bioavailability of drugs, especially drugs with a narrow therapeutic range.

The aim of this study was to examine the influence of various drugs and nutrients on the expression levels of intestinal drug transporters PEPT1, MDR1, MRP2, and MRP3. Furthermore, we also examined the effects on expression of the enzyme CYP3A4, because of the possible interaction between drug efflux and metabolism via coordinate regulation of CYP3A4 and MDR1 expression (4). We used *in vitro* intestinal cell lines to screen the effects of a wide variety of drugs and diets, even though it is not yet known which cell lines are the most suitable for this purpose. Caco-2 cells have been most widely used as a human small intestinal model in research on drug transport, and show a good correlation with normal human jejunum in terms of the expression of ATP-binding cassette transporters (5). Therefore, here we used Caco-2 cells to examine the influence of drugs, nutrients and components of the culture medium on the expression levels of intestinal transporters. LS180 cells were also used for comparison, because the up-regulation of MDR1 via a steroid xenobiotic receptor (SXR)-mediated process has been well studied in this cell line (4).

MATERIALS AND METHODS

Materials

[^{3}H]Digoxin (703.0 GBq/mmol) and [^{14}C]mannitol (2035 GBq/mol) were purchased from Perkin-Elmer™ Life Sciences Inc. (Boston, MA, USA). SUPERSCRIPT™ II RNase H$^-$ RT and Oligo(dT)$_{12\text{-}18}$ were purchased from Life Technologies (Rockville, MD, USA). LC-FastStart DNA Master SYBR Green I was purchased from Roche Diagnostics GmbH (Mannheim, Germany). Herring sperm DNA was purchased from Promega Co. (Madison, WI, USA). C219 monoclonal antibody was purchased from Signet Pathology Systems, Inc. (Dedham, MA, USA). Other reagents were of analytical grade.

Cell Culture

Caco-2 cells were obtained from American Type Culture Collection (Manassas, VA, USA), and those between passages 30 and 60 were used. Cells were grown as described previously (6). Cells were seeded at a density of 6.3×10^4

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ABBREVIATIONS: ATCC, American Type Culture Collection; ATRA, *all-trans* retinoic acid; HBSS, Hanks'-balanced salt solution; PCR, polymerase chain reaction; TEER, transepithelial electrical resistance.

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cells/cm^2 onto 6-well multidishes (NUNC, Roskilde, Denmark) coated with collagen. On day 14 postseeding, the culture medium was replaced with fresh medium with or without a test compound, and the medium was changed every day thereafter. On day 17, total RNA was extracted from the cells.

LS180 cells were obtained from Dainippon Pharm. Co. (Tokyo, Japan), and used between passages 49 and 60. Cells were grown on 10-cm diameter culture dishes at 37°C in a humidified atmosphere with 5% CO_2. The culture medium consisted of Eagle's minimum essential medium (ICN Biomedicals Inc., Aurora, OH, USA), containing 10% FBS. Cells were seeded at a density of 2.0×10^6 cells/well onto 6-well multidishes (NUNC, Roskilde, Denmark). On day 4 postseeding, the culture medium was replaced with fresh medium, with or without a test component. On day 5, total RNA was extracted from the cells.

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Total RNAs were extracted from Caco-2 and LS180 cells using a RNeasy Mini Kit (QIAGEN). Five micrograms of total RNA was reverse-transcribed using SUPERSCRIPT™ II RNase H$^-$ RT.

As a standard for quantitation, cDNA was synthesized by means of the PCR method using suitable sets of primers to amplify MDR1, MRP2, MRP3, CYP3A4 and beta-actin (Table I). Specific primers for these genes were as follows: MDR1 (AF016535) sense: 5′-GCTCCTGACTATGCCAAAGC-3′ and antisense: 5′-ATTAGGCCTTCCGTGCTGTA-3′, MRP2 (NM_00392) sense: 5′-CTCATTCAGACGACCATCCA-3′ and antisense: 5′-GGCTGCCGCACTCTATAATC-3′, MRP3 (NM_020038) sense: 5′-GACTTCCAGTGCTC AGAGGG-3′ and antisense: 5′-TGTCAGTCTCCAGGTCGATG-3′, CYP3A4 (AF182273) sense: 5′-AAGTCGCCTCGAAGATACAC-3′ and antisense: 5′-TGCAGTTTCTGCTGGAC ATC-3′, SXR (NM_33013) sense: 5′-TTGTTCGGCATCACAG GTAG-3′ and antisense: 5′-CTTGCCTCTCTGATGGTCCT-3′, beta-actin (NM_001101) sense: 5′-AAGAGATGGCCACGG CTGCT-3′ and antisense: 5′-TCCTTCTGCATCCTGTCGGCA-3′. Reaction mixtures were subjected to 35 cycles of PCR, then the PCR products were electrophoresed on a 2% agarose gel and stained with ethidium bromide. The amount of each PCR product was measured with an AE-6955 Light Capture instrument (ATTO Co., Tokyo, Japan) using a Low DNA Mass™ Ladder (Gibco BRL) as a standard marker. The control cDNA thus obtained was diluted with 10 ng/mL herring sperm DNA solution to give a final concentration of 10^6, 10^5, 10^4, 10^3, 10^2, or 10 copies/μL.

Table I. Effects of Glucose on Gene Expression in Caco-2 Cells

	Glucose conc. (g/L)			
Gene	4.5	1.0	0.5	0
PEPT1	100.0 ± 14.7	88.6 ± 10.8	186.0 ± 13.9	161.7 ± 12.9
MDR1	100.0 ± 9.8	70.1 ± 7.7	479.9 ± 40.2*	1021.2 ± 19.6*
MRP2	100.0 ± 19.9	74.0 ± 3.6	261.2 ± 25.9*	183.0 ± 17.8*
MRP3	100.0 ± 22.0	40.7 ± 20.7	167.8 ± 29.3	161.2 ± 33.8
CYP3A4	100.0 ± 63.5	60.3 ± 38.4	65.5 ± 15.4	96.7 ± 0.1
Beta-actin	100.0 ± 4.0	109.2 ± 11.6	632.3 ± 29.1*	766.8 ± 22.1*

Note: The gene expression was quantified after incubation of 14-day cultured Caco-2 cells for 24 h in medium containing various concentrations of glucose (4.5, 1.0, 0.5, and 0 g/L). Each column represents the mean ± SEM (n = 3).
* $p < 0.01$ vs. control.

Dilution of Sample cDNA Solution

The cDNA sample solutions were diluted with 10 ng/mL herring sperm DNA solution to give a final total RNA concentration of 1, 5, or 20 ng/μL. The three dilutions were subjected to real-time PCR and the obtained values were expressed in the unit of ng/μL total RNA. The gene expression was normalized using equation 1, where E, E_{gene}, $E_{\text{beta-actin}}$, and $E_{\text{beta-actin(control)}}$ are normalized mRNA expression, the apparent expression of the corresponding gene, beta-actin mRNA expression in the respective sample, and the value of beta-actin mRNA expression of the control, respectively.

$$E = \frac{E_{\text{gene}}}{E_{\beta\text{-actin}}/E_{\beta\text{-actin(control)}}} \quad (1)$$

Western Blotting

Caco-2 cells were treated for 72 h with culture medium containing 10 μM all-*trans* retionic acid (ATRA) or depleted of glucose. Protein expression of P-gp was examined by a Western blotting method using monoclonal antibody C219 against P-gp as described previously (7), Quantitative analysis was done with a cooled CCD camera equipment, and an AE-6955 Light Capture instrument (ATTO Co., Tokyo, Japan).

Transport Experiments

Caco-2 cells were seeded on microporous polycarbonate membrane filters (Transwell™ 3402, Costar) at a cell density of 6.3×10^4 cells/cm^2. The culture medium was changed every other day after confluence. On day 21 postseeding, the culture medium was replaced with fresh medium with or without various constituents, and thereafter the medium was changed every day. On day 24, transport of [^{3}H]digoxin and [^{14}C]mannitol across the monolayers was examined as described previously (6).

The digoxin permeability ratio was calculated by using equation 2, where P and M represent the permeability and molecular weight, respectively.

$$\text{Ratio} = \frac{P_{\text{digoxinB–A}} \times \sqrt{M_{\text{mannitol}}} - P_{\text{mannitolB–A}} \times \sqrt{M_{\text{digoxin}}}}{P_{\text{digoxinA–B}} \times \sqrt{M_{\text{mannitol}}} - P_{\text{mannitolA–B}} \times \sqrt{M_{\text{digoxin}}}} \quad (2)$$

RESULTS

Effects of Culture Conditions on Transporter Gene Expression in Caco-2 and LS180 Cells

We examined the effects of culture medium components, including fetal bovine serum (FBS), glutamine and glucose, and some nutrients, i.e., Gly-Gln, L-lysine, quercetin and ascorbic acid as dipeptide, amino acid, flavonoid, and vitamin model compounds. Furthermore, we also examined the effects of drugs, such as ATRA, rifampin, paclitaxel, and progesterone.

Housekeeping genes were used as internal standards. We chose two housekeeping genes with different physiologic

Table II. Quantification of mRNA Expression in Caco-2 and LS180 Cells Cultured under Various Conditions

A	Caco-2 cells					LS180 cells		B	LS180 cells	
Culture conditions	PEPT1	MDR1	MRP2	MRP3	CYP3A4	MDR1	CYP3A4	Culture conditions	MDR1	CYP3A4
Control	38.7 ± 2.6	71.7 ± 0.6	540.9 ± 72.5	240.9 ± 51.7	0.4 ± 0.0	70.8 ± 4.6	0.3 ± 0.0	Control	43.2 ± 2.5	0.3 ± 0.1
FBS(−)	36.5 ± 0.9	87.7 ± 29.0	327.2 ± 10.2	208.5 ± 104.3	0.9 ± 0.4	123.4 ± 5.9*	1.3 ± 0.2*	Amitriptyline	54.8 ± 7.8	0.2 ± 0.0
Glutamine(−)	32.4 ± 6.1	97.4 ± 7.0	476.5 ± 112.4	205.4 ± 85.4	1.2 ± 0.0*	52.4 ± 3.9	0.4 ± 0.0	Amiodarone	128.0 ± 6.9*	0.6 ± 0.1*
Gly-Gln (4 mM)	41.6 ± 0.7	86.7 ± 3.1	510.4 ± 56.6	166.8 ± 24.4	0.3 ± 0.1	69.4 ± 1.8	0.3 ± 0.1	Coumestrol	37.9 ± 4.7	0.4 ± 0.0
L-Lysine (20 mM)	30.4 ± 1.0	107.3 ± 3.9	558.8 ± 91.9	222.8 ± 43.1	2.0 ± 0.3*	133.1 ± 1.8*	0.6 ± 0.2	Doxorubicin	54.1 ± 4.3	2.1 ± 0.3*
Ascorbic acid (10 μM)	44.8 ± 2.9	92.4 ± 3.0	738.7 ± 183.0	334.5 ± 33.9	1.4 ± 0.3*	90.0 ± 6.7	0.4 ± 0.0	Fluoxetine	59.8 ± 1.1	0.2 ± 0.0
Control (+0.1%DMSO)	30.5 ± 1.9	84.2 ± 6.8	444.0 ± 29.1	147.0 ± 7.1	0.4 ± 0.1	70.4 ± 2.2	0.2 ± 0.0	Imipramine	50.1 ± 4.6	0.2 ± 0.0
ATRA (10 μM)	23.7 ± 2.2	275.6 ± 37.2*	687.2 ± 159.6	270.5 ± 49.5	1.1 ± 0.1*	285.8 ± 48.7*	0.8 ± 0.1*	Midazolam	102.8 ± 11.4*	0.5 ± 0.1
Rifampin (10 μM)	31.4 ± 2.9	71.4 ± 10.7	503.8 ± 90.1	223.0 ± 54.2	0.8 ± 0.5	300.5 ± 17.6*	1.2 ± 0.1*	Nifedipine	153.7 ± 29.5*	0.7 ± 0.1*
Paclitaxel (10 μM)	31.4 ± 2.9	98.5 ± 12.1	349.4 ± 125.8	119.9 ± 8.1	0.2 ± 0.0	204.6 ± 5.7*	0.8 ± 0.2*	Nitrendipine	205.0 ± 9.5*	1.1 ± 0.2*
Progesterone (10 μM)	32.2 ± 2.6	93.2 ± 9.0	408.8 ± 36.3	213.9 ± 17.3	0.3 ± 0.0	124.3 ± 4.3*	0.7 ± 0.3	Rhodamine123	57.3 ± 4.5	0.3 ± 0.0
Quercetin (10 μM)	66.8 ± 4.6*	127.2 ± 25.0	481.5 ± 17.4	379.0 ± 70.5*	0.9 ± 0.3	116.2 ± 11.7	0.4 ± 0.1	Vinblastine	155.5 ± 15.5*	0.1 ± 0.0

Note: Data are expressed as the beta-actin normalized copy number of transcripts per nanogram of total RNA. Drugs shown in the column headed culture conditions, i.e., ATRA, rifampin, paclitaxel, progesterone, and quercetin were dissolved in DMSO and used at a final concentration of 0.1% DMSO. Caco-2 cells at day 14 of cultivation were incubated with the compounds for 72 h, whereas LS180 cells at day 4 were incubated for 24 h. Drugs shown in part B were dissolved in DMSO to give a final concentration of 10 μM in 0.1 % DMSO. Each column represents the mean ± SEM ($n = 3$).

* $p < 0.01$ vs. control.

functions, i.e., beta-actin and beta-2 microglobulin. The expression levels of these genes were consistent under all the conditions examined (data not shown), except for glucose-depleted medium, in which the expression was greatly increased (Table I). Therefore, normalization was performed with respect to beta-actin in each case, using equation (1), except in the case of glucose depletion.

Caco-2 cells showed a remarkable increase in mRNA expression level of MDR1 (10-fold) and beta-actin (7-fold) in response to the decrease of glucose concentration in the culture medium, whereas those of PEPT1 (1.5-fold), MRP2 (2-fold),and MRP3 (1.5-fold) showed small increases (Table I). Treatment with quercetin increased the expression of PEPT1, whereas ATRA induced the expression of MDR1 (Table IIA). CYP3A4 expression tended to increase in the glutamine-free condition, whereas it was increased by L-lysine and ATRA, and also slightly by ascorbic acid (Table IIA). No change was observed under other conditions.

In LS180 cells, we confirmed the upregulation of MDR1 by rifampin and paclitaxel, and a similar increase was seen with ATRA (Table IIA). CYP3A4 expression was also increased by paclitaxel, rifampin, and ATRA, and in the absence of fetal bovine serum (Table IIA). To confirm the difference of nuclear receptor expression between these two cell lines, SXR expression was determined with an RT-PCR method. SXR was detected in LS180 cells, but not in Caco-2 cells (Fig. 1).

P-gp Expression and Function in Caco-2 Cells

The expression of MDR1 was increased more than 3-fold in the presence of ATRA (Table II), and about 10-fold in the glucose-free condition (Table I). Apparent increases in the expression of PEPT1, MRP2 and MRP3 in response to the decrease of glucose concentration may not be significant, since the expression of beta-actin was similarly increased. Therefore, protein expression was examined using Western blotting and transport experiments were performed to confirm the change of P-gp function.

P-gp protein expression was increased in glucose-free medium (about 1.3-fold) and in the presence of ATRA (about 1.3-fold) for 72 h (Fig. 2A). To test the functional change of P-gp, transcellular transport of [^{3}H]digoxin (a substrate of P-gp) and [^{14}C]mannitol (a paracellular transport marker) was examined. Because the transepithelial electrical resistance decreased markedly (about 70%, data not shown) during glucose depletion for 72 h, glucose depletion was performed for 24 h in the transport experiments (transepithelial electrical resistance did not change; data not shown). The B (basolateral) to A (apical) permeability ratio of [^{3}H]digoxin corrected for paracellular transport using equation 2 was increased significantly in glucose-free medium and slightly in the presence of ATRA (Fig. 2B, insert graph). The lack of any significant change of the permeability ratio of digoxin in response to 0.5 g/L glucose may result from the small magnitude of the increase of P-gp protein.

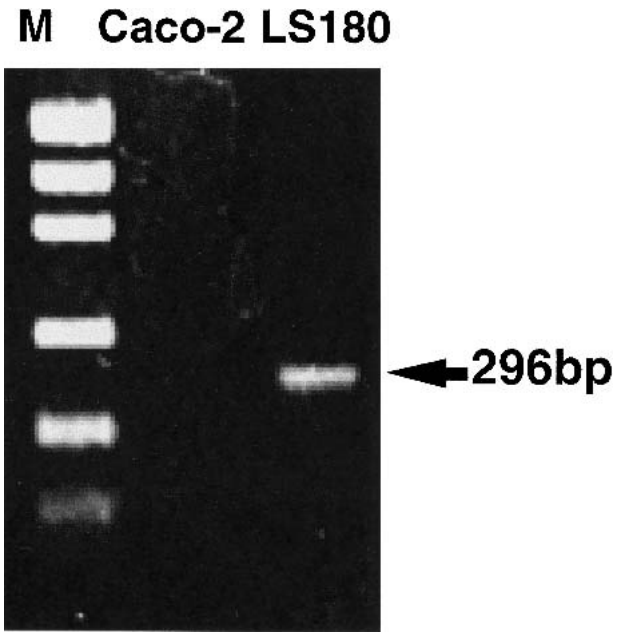

Fig. 1. Expression of steroid xenobiotic receptor in Caco-2 cells and LS180 cells. Total RNA from cultured Caco-2 cells and LS180 cells was subjected to reverse-transcription polymerase chain reaction (PCR) using primer pairs specific for the steroid xenobiotic receptor gene. The reverse-transcription PCR products were analyzed by 2% agarose gel electrophoresis and stained with ethidium bromide. The arrowhead indicates the predicted size (296 bp) for the PCR product.

Effects of Drugs on MDR1 and CYP3A4 Expression in LS180 Cells

There is an interaction between drug metabolism by CYP3A4 and efflux by MDR1, since paclitaxel is metabolized by CYP3A4 and effluxed by MDR1. Therefore, we examined expression of MDR1 and CYP3A4 in LS180 cells after treatment with substrates of P-gp or ligands of SXR for 24 h. MDR1 expression was induced by amiodarone, midazolam, nifedipine, nitrendipine and vinblastine, while little change was observed under other conditions. CYP3A4 expression was induced by amiodarone, doxorubicin, nifedipine and nitrendipine (Table IIB).

DISCUSSION

Some work has been performed on the regulation of transporter expression by nutrients. For example, the amino acid L-lysine (3) increased the activity of rat PEPT1 promoter in Caco-2 cells, whereas in the present study, no increase in the expression of human PEPT1 was observed (Table II). An amino acid-responsive element was detected at approximately –277 bp to –271 bp upstream of the rat PEPT1 gene but was not present up to about –2 kbp upstream of the human PEPT1 gene (3). We searched for sequences corresponding to amino acid-responsive element using the Celera Discovery System™ and found this sequence at –1969 bp, –4861 bp, –6042 bp, –6677 bp, –6986 bp, and –8356 bp from the transcription initiation site of the human PEPT1 gene. Thus, there appears to be a species difference between the rat and human PEPT1 sequences, which may account for the difference in response to L-lysine.

Glutamine and glucose are major energy sources of cells and FBS provides some growth factors. Cultivation with glutamine-reduced medium decreased the expression of MDR1 in multicellular prostate tumor spheroids, DU-145 cell line (8). We found no change of drug transporter expression in

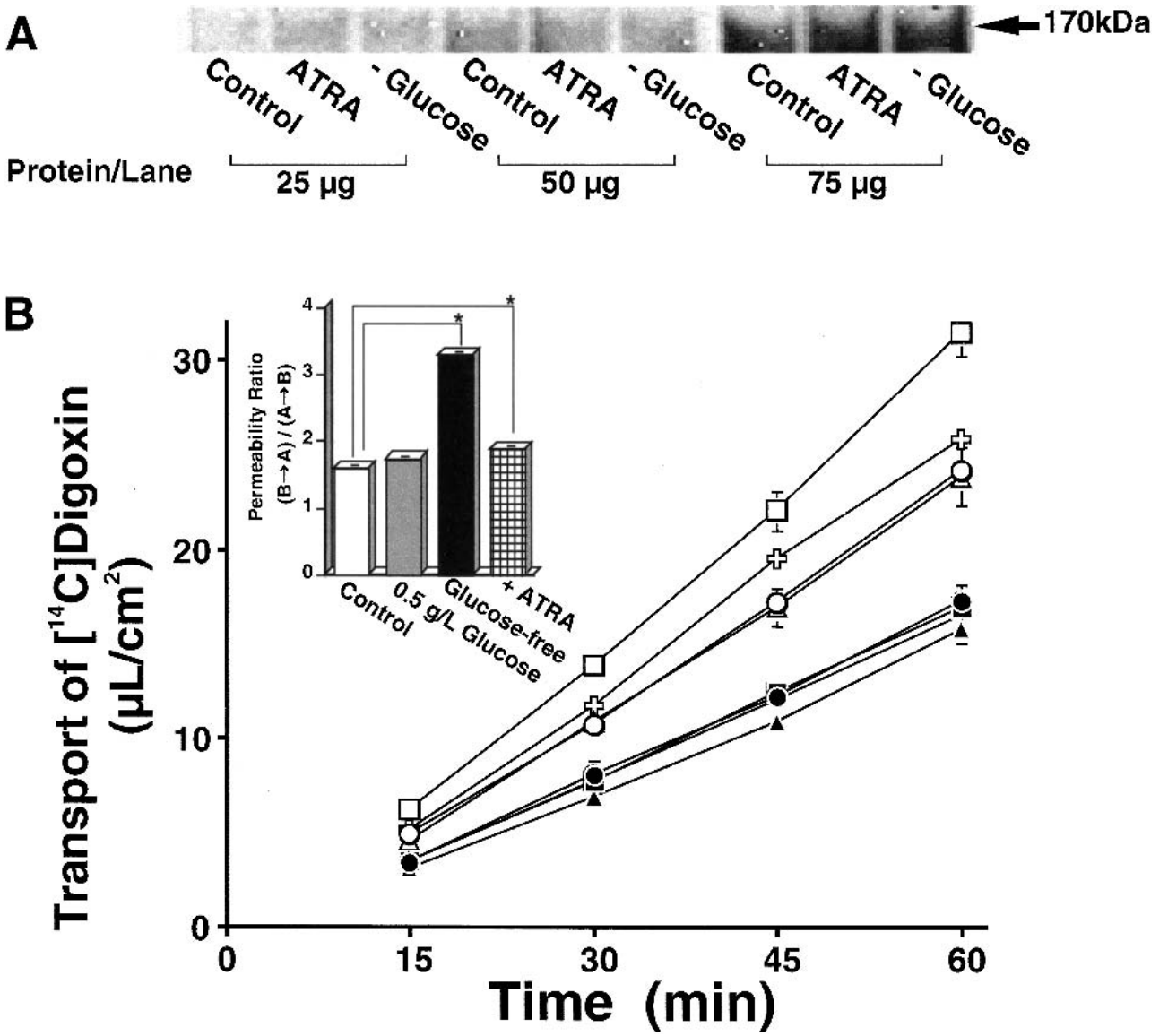

Fig. 2. P-gp expression and function in Caco-2 cells. A, Caco-2 cells were incubated in glucose-free or all-*trans* retionic acid (ATRA)-supplemented medium for 72 h. Membrane proteins were extracted with 4% sodium dodecyl sulfate and subjected to Western blot analysis using anti-P-gp monoclonal antibody C219. Horseradish peroxidase-linked anti-mouse IgG was used as the secondary antibody (NEN™ Life Science Products, Inc., Brea, CA, USA), and the protein was detected by the enhanced chemiluminescence detection method using the ECL-plus western-blotting detection system (Amersham Pharmacia Biotech, Buckinghamshire, UK). Quantitative analysis was performed by using a cooled CCD camera equipment with a Light Capture apparatus (ATTO, Co., Tokyo, Japan). B, Caco-2 cell monolayers were incubated with 0.5 g/L glucose-containing, glucose-free, or ATRA-supplemented culture medium for 24 h, and the permeability of [^{3}H]digoxin (11.3 nM) and [^{14}C]mannitol was measured at 37°C for 60 min in Hanks' balanced salt solution buffer ($pH_{apical}/pH_{basolateral}$ = 7.4/7.4). Open and closed symbols represent apical to basolateral (A-B) and basolateral to apical (B-A) transport, respectively. Circle, square, triangle, and cross symbols represent ordinary (control), glucose-free, 0.5 g/L glucose-containing, and ATRA-supplemented culture media, respectively. The inset represents the permeability ratio normalized with respect to that of mannitol. Each point represents the mean ± SEM (n = 3). *Significantly different from the control permeability ratio by Student's *t* test ($p < 0.05$).

Caco-2 and LS180 cells grown in glutamine-free or fetal bovine serum-free medium (Table IIA). It is possible that different transcriptional factors are involved in different cell lines.

However, we found that glucose depletion significantly increased the expression of MDR1, in contrast to a report that MDR1 mRNA levels did not change after glucose starvation (9). Glucose-induced hyperaccumulation of cAMP and defective glucose repression in yeast were reported to reduce the activity of cAMP-dependent protein kinase (10). Furthermore, through inhibition of cAMP-dependent protein kinase activity, MDR1 expression was blocked in doxorubicin-resistant MCF-7 breast cancer cells (11). In this study, exposure to glucose-free and 0.5 g/L glucose media increased the expression of MDR1 in Caco-2 cells (Table I). This may involve a cAMP-dependent mechanism, although this remains to be confirmed.

Nuclear receptors are considered to be involved in gene regulation. ATRA, one of the active metabolites of vitamin A, is a specific ligand of RAR, and RAR alpha gene expres-

sion affects MDR1 expression and P-gp function in human and rodent cell lines (12). In this study, we confirmed that ATRA increases the MDR1 expression level in both Caco-2 and LS180 cells.

SXR is activated by many xenobiotics, and coordinately regulates multiple xenobiotic clearance pathways (metabolism and excretion) in the intestine and the liver (13). Activation of SXR by rifampin and paclitaxel was considered to increase the expression of MDR1 and CYP3A4 (4), although no change of MDR1 expression in Caco-2 cells was observed in this study (Table II). The reason for this may be the absence of SXR in Caco-2 cells (Fig. 1). Furthermore, rifampin activates SXR, a human typical pregnane X receptor (PXR) and rabbit PXR, but has virtually no activity on mouse or rat PXR (14,15). Therefore, animal models such as rat and mice, and human SXR-null cell lines, are not suitable for studies of regulatory processes mediated by SXR.

In LS180 cells, we found that MDR1 and CYP3A4 expression was increased by amiodarone, doxorubicin, nifedipine, nitrendipine, rifampin and paclitaxel, while progesterone, vinblastine and midazolam increased the expression of MDR1 (Table II). Nitrendipine, paclitaxel, rhodamine123, vinblastine, and doxorubicin are known to be P-gp substrates. Vinblastine, nifedipine, midazolam, and paclitaxel are metabolized by CYP3A4. Rifampin, paclitaxel, and nifedipine are reported to be ligands of SXR. Thus, the expression of both MDR1 and CYP3A4 may be at least partly regulated via similar mechanisms. P-gp and CYP3A4 are coordinately increased by many xenobiotics and clinically administered drugs that are substrates of MDR1 or CYP3A4. Therefore, drug interactions caused by alterations in the expression of transporters and enzymes should be considered as a cause of altered pharmacokinetics.

Our findings here suggest that bioavailability of various drugs may be altered by changes in the expression of intestinal transporters or metabolizing enzyme by coadministered drugs. This would be especially important for drugs with a narrow therapeutic range. Because SXR is involved in some expression-regulatory processes, and Caco-2 cells lack SXR, we consider that LS180 cells as well as Caco-2 cells should be used for the study of the regulation of intestinal transporters.

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